

Project Report: The evolution and diversity of ancient CO₂-fixation pathways in anaerobic and extremophilic microorganisms: Clues to the early evolution of life on Earth

Project Investigator:

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Project Progress

This project report reports on the accomplishments of the project "The evolution and diversity of ancient CO₂ -fixation pathways in anaerobic and extremophilic microorganisms: Clues to the early evolution of life on Earth" as part of NNA04CC04A "From Early Biospheric Metabolisms to the Evolution of Complex Systems" (Stefan Sievert – Co-PI, Woods Hole Oceanographic Institution (WHOI)).

We have selected a total of 13 bacteria or archaea on the basis of having, or being suspected of having, enzymes of the reductive tricarboxylic acid cycle (RTCA). Most of the microorganisms were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) as active or freeze-dried cultures. Except for two organisms, all are thermophiles that have optimum growth temperatures from 60–100 °C. Incubations at 8 different temperatures within this range were required. Ten different growth media were prepared in serum bottles to re-establish growing cultures from the purchased ones. Since enzymes involved in autotrophic CO₂ fixation can be oxygen sensitive, a procedure to harvest and store cells under anoxic conditions was developed and employed. Cells for nucleic acid extraction were also concentrated and stored by the same method. So far, nucleic acids have been extracted from eight cultures. We have also received deoxyribonucleic acid (DNA) from Constantino Vetriani (Rutgers University) and Ken Takai (JAMSTEC, Japan), with whom we are collaborating on these questions. All these organisms either belong to the *ε*-proteobacteria or the *Aquificales*. Using a PCR protocol established in our laboratory we have so far successfully amplified ACL genes from eight cultures. In addition, we are also in the process of obtaining ACL sequences from deep-branching eukaryotes to reconstruct the evolutionary history of this enzyme. So far we have amplified ACL from representatives from seven groups.

We now have biochemical and genetic evidence for the operation of the RTCA in two members of the *ε*-proteobacteria, and in *Desulfurobacterium autotrophicum* (*Aquificales*). Bacteria of the *ε*-proteobacteria have been identified as very important members of the microbial community at

deep-sea hydrothermal vents. However, no information exists as to what CO₂ –fixation pathway might be used by ϵ –proteobacteria or *Desulfurobacterium*. This work is presently being prepared for submission to the *Journal of Bacteriology*.

Highlights

- ϵ –proteobacteria use the reductive TCA cycle for autotrophic fixation of CO₂.
 - ◆ In recent years, the ϵ –subdivision of the proteobacteria has received increased attention as small subunit ribosomal RNA genes (ssrRNA) phylogenetically associated with ϵ –proteobacteria have been frequently obtained from a variety of environments, including hydrothermal vents, the oxic–sulfidic interfaces of Cariaco Basin and the Black Sea, and oilfields indicating their importance in these systems. Recently, several chemolithoautotrophic ϵ –proteobacteria have been isolated. However, no direct information, i.e., biochemical and genetic, existed prior to our work that autotrophic carbon fixation might be used by these bacteria.
- Autotrophic fixation of CO₂ through the reductive TCA cycle might be more significant and widespread than previously thought.
 - ◆ ϵ –proteobacteria have been identified as a major, if not dominant, component of microbial communities in a variety of extreme habitats (see above). There is data that suggest that many of these organisms could potentially be autotrophs, obtaining energy by oxidation of reduced sulfur compounds and hydrogen with dissolved free oxygen or nitrate, which makes it likely that these organisms contribute significantly to primary production in these systems.
- ACL will prove useful as a functional gene marker to assess the diversity and abundance of organisms utilizing this pathway for autotrophic carbon fixation in the environment.
 - ◆ The results obtained so far show that the phylogeny obtained based on ACL sequences is largely congruent with phylogenetic trees based on 16S rRNA or 18S RNA. However, clearly more sequence data is needed to further confirm this result.

Roadmap Objectives

- **Objective No. 3.2:** Origins and evolution of functional biomolecules
- **Objective No. 4.1:** Earth's early biosphere